

Claims

1. Method for detecting and/or purifying substances selected from
5 proteins, biomolecules, complexes of proteins or biomolecules, subunits thereof, cell components, cell organelles and cells comprising the steps:

(a) providing an expression environment containing one or more
heterologous nucleic acids encoding one or more polypeptides
10 and/or one or more subunits of a biomolecule complex, the polypeptides or subunits being fused to at least two different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A,

(b) maintaining the expression environment under conditions that
15 facilitate expression of the one or more polypeptides or subunits in a native form as fusion proteins with the affinity tags,

(c) detecting and/or purifying the one or more polypeptides or
subunits by a combination of at least two different affinity
20 purification steps each comprising binding the one or more polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the one or more polypeptides or subunits from the support material after substances not bound to the support
25 material have been removed.

2. Method for detecting and/or purifying biomolecule and/or protein
complexes, comprising the steps:

(a) providing an expression environment containing one or more
30 heterologous nucleic acids encoding at least two subunits of a biomolecule complex, each being fused to at least one of

different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A,

- (b) maintaining the expression environment under conditions that facilitate expression of the one or more subunits in a native form as fusion proteins with the affinity tags, and under conditions that allow the formation of a complex between the one or more subunits and possibly other components capable of complexing with the one or more subunits,
- (c) detecting and/or purifying the complex by a combination of at least two different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after substances not bound to the support material have been removed.

3. Method according to claim 1 or 2, wherein between the one or more polypeptides or subunits and one or more of the affinity tags a specific proteolytic cleavage site is present in the fusion protein which facilitates the removal of one or more of the affinity tags.

4. Method according to claim 3, wherein the specific proteolytic cleavage site is an enzymatic cleavage site.

5. Method according to claim 4, wherein the specific proteolytic cleavage site is the cleavage site for TEV protease NIA.

6. Method according to claim 3, 4 or 5, wherein the proteolytic cleavage site is used to cleave the polypeptide or subunit in step (c) from the IgG binding domain of Staphylococcus protein A bound to the support material.

7. Method according to claim 6, wherein the affinity purification of step (c) comprises:
- (i) binding the one or more polypeptides or subunits via the one or more IgG binding domains of *Staphylococcus* to a support material capable of specifically binding the latter, removing substances not bound to the support material and separating the one or more polypeptides or subunits from the support material by cleaving off the IgG binding domains via the specific proteolytic cleavage site, and
 - (ii) binding the polypeptide or subunit via another affinity tag to a second support material capable of specifically binding the latter, removing substances not bound to the support material and separating the polypeptide or subunit from the support material.
8. Method according to claim 7, wherein step (ii) is carried out before step (i).
9. Method according to one of the previous claims, wherein the fusion protein contains a second specific proteolytic cleavage site for the removal of one or more of the other affinity tags.
10. Method according to one of the previous claims, wherein one of the affinity tags consists of at least one calmodulin binding peptide.
11. Method according to claim 10, wherein a chemical agent is used to separate the one or more polypeptides or subunits from the support material.
12. Fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein

one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A.

- 5 13. Fusion protein according to claim 12, wherein it additionally contains a specific proteolytic cleavage site.
14. Nucleic acid coding for a fusion protein according to claim 12 or 13.
- 10 15. Vector comprising a nucleic acid according to claim 14 under the control of sequences facilitating the expression of a fusion protein according to claim 12 or 13.
- 15 16. Vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least two different affinity tags one consisting of one or more IgG binding domains of Staphylococcus aureus protein A, and at least one polynucleotide linker for the insertion of further nucleic acids.
- 20 17. Vector comprising heterologous nucleic acid sequences in form of two or more cassettes each comprising at least one of different affinity tags one consisting of one or more IgG binding domains of Staphylococcus aureus protein A, and at least one polynucleotide linker for the insertion of further nucleic acids.
- 25 18. Cell containing a nucleic acid according to claim 14 or a vector according to claim 15.
- 30 19. Reagent kit comprising a nucleic acid according to claim 14 or a vector according to claim 15, 16 or 17 for the expression of a fusion protein according to claim 12 or 13 and support materials each capable of specifically binding one of the affinity tags.

20. Reagent kit according to claim 19 additionally comprising at least one chemical agent for separating one of the affinity tags from its support material and/or a specific chemical proteolytic agent and/or specific protease capable of cleaving the fusion protein.

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21. Use of the method according to one of claims 1 to 11 for the detection and/or purification of substances capable of complexing with the fusion protein.

- 10 22. Use of the method according to one of claims 1 to 11 for the detection and/or purification of cells and/or cell organelles expressing the fusion protein on their surface.

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